Published in final edited form as:

Neuroimage. 2012 August 15; 62(2): 736–742. doi:10.1016/j.neuroimage.2012.01.013.

A review of the development of Vascular-Space-Occupancy (VASO) fMRI

Hanzhang Lu^a and Peter van Zijl^{b,c}

^aAdvanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

^bDepartment of Radiology, Johns Hopkins University, Baltimore, MD 21205, United States

^cF.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD 21205, United States

Abstract

Vascular-Space-Occupancy (VASO) fMRI is non-invasive technique to detect brain activation based on changes in Cerebral Blood Volume (CBV), as opposed to conventional BOLD fMRI, which is based on changes in blood oxygenation. This technique takes advantage of the T1 difference between blood and surrounding tissue, and uses an inversion recovery pulse sequence to null blood signal while maintaining part of the tissue signal. The VASO signal intensity can thus be considered proportional to 1-CBV. When neural activation causes CBV to increase, the VASO signal will show a decrease, allowing the detection of activated regions in the brain. *Activation-induced changes in VASO signal, $\Delta S/S$, are on the order of -1% .* Absolute quantification of ΔCBV requires additional assumptions on baseline CBV and water contents of the parenchyma and blood. The first VASO experiment was conducted approximately ten years ago. The original goal of nulling the blood signal was to isolate and measure extravascular BOLD effects, thus a long TE of 50 ms was used in the inversion recovery experiment. Instead of a positive signal change, a slight decrease in signal was observed, which became more pronounced when TE was shortened to 10 ms. These findings led to the hypothesis of a CBV signal mechanism and the development of VASO fMRI. Since its discovery, VASO has been validated by comparison with MION-CBV studies in animals and has been used in humans and animals to understand metabolic and hemodynamic changes during brain activation and physiologic challenges. With recent development of more sensitive VASO acquisitions, the availability of arterial-based VASO sequences, and improvement in spatial coverage, this technique is finding its place in neuroscience and clinical studies.

Keywords

Vascular-Space-Occupancy (VASO); fMRI; cerebral blood volume (CBV); neurovascular coupling; BOLD

© 2011 Elsevier Inc. All rights reserved

Corresponding Author: Hanzhang Lu, Ph.D. Advanced Imaging Research Center UT Southwestern Medical Center 5323 Harry Hines Blvd. Dallas, TX 75390 Hanzhang.Lu@UTSouthwestern.edu Tel: 214-645-2761 Fax: 214-645-2744.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Over the past ten years, our group at the F.M. Kirby research Center at Kennedy Krieger Institute and Johns Hopkins University has developed a technique to use activation-related changes in Cerebral Blood Volume (CBV) as a contrast mechanism to conduct functional MRI. This technique, dubbed Vascular-Space-Occupancy (VASO) fMRI, can non-invasively and dynamically measure CBV changes in humans (Lu et al., 2003). VASO-fMRI is complementary to oxygenation-based BOLD and CBF-based ASL fMRI signals and, when applied conjunctively, can improve our understanding of neurovascular coupling and help obtain quantitative interpretation of the BOLD signal in terms of oxygen metabolism and the oxygen extraction fraction. The present article provides a brief review of the initial development of VASO fMRI, the opportunities it brings, and its limitations.

An unexpected finding which led to the conjecture of CBV-based fMRI

Around 2001, the fMRI community was actively working on developing a quantitative understanding of the BOLD signal, with the hope that the BOLD percentage signal change could be directly linked to oxygenation and, potentially, cerebral metabolism (Logothetis et al., 2001). Since deoxyhemoglobin in the blood can affect the transverse relaxation of both the blood signal itself and the surrounding tissue, the relationship between oxygenation and BOLD is rather complicated (Boxerman et al., 1995; Buxton et al., 1998; Davis et al., 1998; Ogawa et al., 1993; van Zijl et al., 1998; Yablonskiy and Haacke, 1994; Zhong et al., 1998). At that time, there was a big debate as to how much of the BOLD signal originates from intravascular (blood) signal and how much from extravascular (tissue) signal (Boxerman et al., 1995; van Zijl et al., 1998; Zhong et al., 1998). We therefore conceived an idea that, if we could make the blood signal “invisible” (or nulled) to MRI, we could then measure the pure tissue signal and, by comparing the signal to the original BOLD signal, estimate the fractional contributions of extra- and intravascular signals to BOLD. A second benefit of measuring only the tissue BOLD signal would be that biophysical modeling of a single (tissue) compartment is considerably simpler than a multi-compartment (tissue, arterial and venous blood) model (Boxerman et al., 1995; Yablonskiy and Haacke, 1994; Zhong et al., 1998). Therefore, it would be more straightforward to link the tissue BOLD signal to quantitative oxygenation values from the modeling perspective, as opposed to the use of total BOLD.

Therefore, we set out to search for a method to null the blood signal yet maintaining (at least some) tissue signal. Because blood T1 was well known to be longer than tissue T1 (Duck, 1990; Hoogenraad et al., 2001; Lu et al., 2002), the use of an inversion recovery sequence with the inversion time (TI) set at the blood nulling point came to mind. We also noted that, since the blood spins in the voxel are continuously replaced by newly arrived fresh spins, a non-slice-selective inversion pulse needed to be used. Another advantage of using T1 properties to separate tissue from blood signals is that the excitation and acquisition modules of the pulse sequence are not restricted and one can maintain the typical BOLD echo times and acquisition schemes (e.g. single-shot gradient-echo EPI) to obtain T2* weighting (for the measurement of extravascular BOLD).

To choose a proper TI to null the blood signal, the T1 of blood needs to be known. The relationship between these two parameters can be written as:

$$1 - 2 \cdot e^{-TI/T_1} + e^{-TR/T_1} = 0 \quad [1]$$

When a long TR is used (e.g. >6 seconds), the equation can be simplified to $TI = T_1 \cdot \ln(2)$. In 2001, most research centers, including ours, were using 1.5 Tesla MRI systems. At this field strength, the values of blood T1 were not completely conclusive (Duck, 1990). Researchers

in the ASL community also needed to use blood T1 for CBF quantification and many of them were assuming values between 1.1–1.2 seconds (Alsop and Detre, 1996). We noted that this assumption was based on values measured from blood samples that were not necessarily under physiologic conditions. One of the most critical conditions for T1 measurements is the temperature, because blood T1 increases approximately linearly with temperature (Lu et al., 2004a). Thus, a measurement conducted at room temperature (25°C) is expected to yield T1 values approximately 200 ms lower than that at body temperature (37°C). We therefore first conducted *ex vivo* experiments on blood samples while controlling their temperature, pH, metHb level, and cell precipitation to be under physiologic conditions. We showed that the blood T1 is in the range of 1.35–1.4 seconds (Lu et al., 2003). Another requirement for our “blood-nulling” idea to work was that both arterial and venous blood needed to be nulled with the same pulse sequence. In other words, blood T1 should be relatively insensitive to blood oxygenation. This is obviously not the case for blood T2 as the T2 dependence on oxygenation was the basis of the BOLD effect. Fortunately, we found that blood T1 was indeed relatively constant at different oxygenation levels with an arterial and venous value of 1355 ms and 1390 ms, respectively (Lu et al., 2003). With these preparation steps completed, we were ready to conduct the human experiments.

We did our first, at that time called IR-fMRI, experiment on a 22 year old male volunteer on October 10, 2001. We used a non-slice-selective inversion recovery sequence with a TI of 920 ms and a TR of 5920 ms. A single slice was acquired as the blood magnetization has only one zero-crossing point in each TR. Importantly, we used a long-TE single-shot gradient-echo acquisition (TE = 50 ms) to achieve optimal BOLD contrast as our original goal was to quantify the extravascular BOLD during blood signal nulling. Our stimulus paradigm was a simple blue-yellow checkerboard visual stimulation with a block design of 29.6 seconds ON interleaved with 29.6 seconds OFF. Each block yielded 5 dynamic images and the ON/OFF blocks were repeated three times. The duration of the scan was about three and a half minutes. For comparison, we performed a conventional BOLD scan with identical parameters but the inversion pulse disabled.

The conventional BOLD scan yielded the typical signal increase of about 3% during stimulation (Figure 1a). For the IR-fMRI scan, we had expected that the signal change would be dampened but would still show a significant increase. To our surprise, a different pattern was noted. We were not able to detect any significant clusters that showed a positive correlation with the stimulus paradigm. When we studied the IR-fMRI signal time courses in the BOLD-activated voxels (detected by the conventional BOLD scan) only, we did not observe a signal increase either. A closer look of the (somewhat noisy) time course (Figure 1b) suggested that the signal might even be showing a ‘NEGATIVE’ change with a correlation coefficient -0.37 with reference to the stimulus paradigm. Increases in CBV appear to be the only reasonable mechanism for such a negative signal change that our data seemed to show. As a simplified explanation, the blood signal is nulled thus the measured signal in terms of parenchymal fraction (with 1 being total parenchyma) is $1 - \text{CBV}$. When CBV increases upon stimulation, the MR signal would show a decrease.

Further development and optimization of CBV-based fMRI

Despite these interesting findings and potential possibility of a new brain mapping mechanism, the time course in Figure 1b was very noisy and far from conclusive. It was even possible that the negative signal change was due to stimulation-related subject motion. Furthermore, the signal decrease was so small that we were not able to detect any significant clusters in voxelwise analysis. This was of course related to the fact that our original goal was to quantify extravascular BOLD effects, for which we had chosen a long TE of 50 ms in order to maximize BOLD sensitivity. However, we concluded that in doing that we created a

scenario in which positive BOLD signal partially cancelled out the negative CBV effect. Fortunately, the CBV contrast was achieved in the magnetization preparation stage of the pulse sequence via inversion recovery and the BOLD contrast was generated in the acquisition stage via proper T2* weighting. Therefore, by adjusting the TE of the sequence, one could modulate the relative weightings of these two effects. In order to highlight the CBV effect, a short TE should be used, while BOLD effects would come in at longer TE.

In the two months following the first experiment, we tested the TE-dependence of the IR-fMRI signal. We used two-echo acquisition with a “short” TE of 25 ms to emphasize CBV contrast and a longer TE of 65 ms to have predominantly BOLD contrast. While the ROI (based on the conventional BOLD activation map) results consistently showed positive correlation at TE = 65 ms and negative correlation at TE = 25 ms, we were still not able to obtain an activation map with the CBV contrast on a voxel-by-voxel basis. We concluded that the TE was still too long. In 2001, due to multiple constraints such as the absence of parallel imaging and low gradient slew rate, the shortest TE for a 64x64 matrix EPI acquisition was around 25 ms. It appeared that considerable BOLD effect was still present at TE of 25 ms, precluding a detection of negative signal change at the voxel level. On December 18, 2001, we therefore tried a half-Fourier approach to the acquisition, which shortened the TE to 11 ms, and this small change proved to be a critical step to allow us to conduct CBV-based fMRI. We were able to obtain voxelwise activation maps using a negative threshold on the cross-correlation value (Figure 2) (*visual stimulation, 29.6 sec ON, 29.6 sec OFF*). With these improvements, it became clear to us that this technique could be utilized as a new contrast for functional brain mapping, but now based on CBV changes (Lu et al., 2003). The most time-consuming task left was to find an appropriate name that, similar to BOLD, would be easy to use and reflect the contrast mechanism. After many rounds of discussion among the authors of this review and our colleagues Xavier Golay and Jim Pekar, we decided to baptize this technique “VAscular-Space-Occupancy” or “VASO” fMRI.

The observed VASO signal change was about -1.5%, an order of magnitude consistent with a CBV contrast mechanism (Lu et al., 2003). As mentioned earlier, the VASO signal intensity is approximately proportional to 1-CBV. Let us consider that CBV at resting state is about 0.05 of the total voxel volume. The VASO signal at resting is then 0.95. Earlier visual activation studies by Belliveau et al. (1991) using Gd-DTPA contrast agent had reported CBV increases of approximately 30% which, using the resting state CBV assumed above, would correspond to a CBV value of 0.065 during activated state. The VASO signal would then be 0.935, corresponding to a signal change between resting and activated states of around -1.6%, in great agreement with our finding. Therefore, the available evidence supported a CBV change to be the origin of the VASO fMRI signal.

In the following several months, we conducted further technical development to confirm our findings. We showed that acquisitions of VASO fMRI can also use spin-echo EPI (with shortest TE possible) or single-shot spiral (with shortest TE possible). The sensitivities using gradient-echo EPI, spin-echo EPI, or spiral acquisitions appeared to be similar as they all minimize the BOLD effects. We also demonstrated that hypercapnia (using breath-hold) and hyperventilation decreased and increased VASO signal, respectively, consistent with their known physiologic effects (Lu et al., 2003). With the upgrade of hardware in the F.M. Kirby Center, especially the availability of parallel imaging and a 3 Tesla system in 2002, we were able to further improve the sensitivity of VASO fMRI. In the following years, several other groups became interested in this technology and made important contributions to its optimization and understanding. These include the combination of VASO with ASL/BOLD acquisitions (Yang et al., 2004), the use of tissue-nulling TIs (Shen et al., 2009; Wu et al., 2008), the use of multiple TIs to simultaneously estimate resting and activated CBV

(Glielmi et al., 2009; Gu et al., 2006), the improved suppression of blood signal (Wu et al., 2007), the use of VASO in calibrated fMRI (Lin et al., 2008a), the effect of CSF contributions on VASO signal (Scouten and Constable, 2007, 2008), the spatial specificity of the signal (Jin and Kim, 2008), and the design of methods for faster acquisition and greater spatial coverage of VASO (Poser and Norris, 2007, 2009, 2011).

Spatial specificity of VASO-fMRI

Vasodilatation associated with neural activation is caused by changes in microenvironment immediately adjacent to the active neurons (Kuschinsky, 1996). Therefore, only blood vessels spatially very close to the activated regions can experience these changes and dilate (Harrison et al., 2002). As a result, VASO fMRI is expected to provide excellent localization, unlike the BOLD effect in which activation patterns may extend to distant regions when blood that has experienced oxygenation changes flows into larger draining veins (Iadecola, 2002; Turner, 2002). This theoretical prediction was confirmed by our early human experiments at 3 Tesla (Figures 3a and b) and later verified at high spatial resolution in the cat brain by Jin and Kim, who convincingly showed that the peak VASO activation signal was observed in layer IV of the cortex (Jin and Kim, 2008) in agreement with MION contrast CBV data in the same animals (Figures 3c and d). BOLD activation patterns, on the other hand, non-specifically covered broader areas of cortical layers and larger vessel regions (Figure 3e). Figure 3f shows a comparison of signal profiles across cortical layers for all three techniques. In a human study comparing retinotopic maps obtained using VASO and BOLD, our group also observed that VASO provided clearer boundaries between adjacent visual cortices (Lu et al., 2005a).

Utility and Applications of VASO fMRI

In the early 2000s, a major interest in the fMRI community was to understand the BOLD contrast mechanism and the physiologic origins of various phases of the BOLD hemodynamic responses. In order to achieve this goal, one needs to separately determine the contributing components of the BOLD effect such as cerebral blood flow (CBF) and CBV. At that time, CBF changes during brain activation could already be measured non-invasively with ASL MRI (Calamante et al., 1999; Detre et al., 1992; Edelman et al., 1994; Golay et al., 1999; Kim, 1995; Wong et al., 1998) and determination of CBV changes in animal models was also possible using long-half-life contrast agents (Mandeville et al., 1998; van Bruggen et al., 1998). These newer contrast agents, however, were not approved for human use due to potential toxicity. Even though the only FDA-approved contrast agent, Gd-DTPA, had previously been used for CBV-based fMRI (Belliveau et al., 1991), this method was not suitable for repeated measures as the researchers would have to wait for the previous bolus to be cleared from the body before applying the next one. Thus, VASO fMRI was the first CBV technique that was non-invasive, fast, and suitable for repeated use in humans in conjunction with other fMRI methods.

Therefore, we combined VASO with BOLD and/or ASL fMRI to obtain quantitative estimates of brain physiologic parameters and for better understanding the BOLD signal. In one study, we used multi-echo VASO fMRI to simultaneously measure CBV changes and extravascular BOLD effects (Lu and van Zijl, 2005). We found that extravascular BOLD constitutes about 47% of the total BOLD at 1.5 Tesla and this fraction increased to 67% at 3 Tesla. Furthermore, by combining information from CBV and BOLD, we could estimate quantitative values of the oxygen extraction fraction (OEF), which reduced by about 40% during activation. In another study, we acquired VASO, BOLD and ASL fMRI in the same session, allowing us to estimate changes in cerebral metabolic rate of oxygen (CMRO₂) (Lu et al., 2004b). The findings showed that brain metabolism remained elevated during a prolonged period following the stimulation, providing a physiologically plausible

explanation for the post-stimulus undershoot commonly observed in BOLD time course and clear evidence of decoupling of CBF and CMRO₂. A more detailed discussion of this undershoot is presented by van Zijl et al. in this Special Issue (van Zijl et al., 2012).

Similar approaches can be used to study CMRO₂ changes during other physiologic conditions. For example, Hua and colleagues employed combined VASO/BOLD/ASL acquisitions to study breath-hold hypercapnia and revealed that CMRO₂ was suppressed during hypercapnia but showed no changes during the post-challenge period, unlike the visual stimulation case (Hua et al., 2011c). In another study, Lin and colleagues used a single scan to acquire VASO, BOLD and ASL signals and showed that metabolic and vascular responses had opposite trends during prolonged visual stimulation of 21 minutes (Lin et al., 2009). Specifically, CMRO₂ increased slowly during continued stimulation, whereas CBF and CBV showed a gradual decrease from the initial peak, presenting an interesting scenario of decoupling between flow and metabolism.

Limitations and future directions for VASO fMRI

Despite the advantages of VASO fMRI in terms of better quantification and higher spatial specificity, this technique is not different from most other MR approaches in that it suffers from a number of limitations and technical development in imaging acquisition and reconstruction has continued in recent years.

One of the limitations is the lower temporal resolution if pure CBV effects need to be studied. In the first VASO study, we used a long TR of ~6 seconds and observed a signal change of 1–1.5%, which was consistent with the expected CBV change of 20–30% upon brain activation (Lu et al., 2003). To speed things up, we tested a shorter TR while of course changing TI accordingly to maintain the blood nulling condition described in Eq. [1]. Although in theory the expected VASO signal change should not be dependent on TR, we observed that it was much more pronounced at shorter repetition times (Lu et al., 2005b). For TR = 3 s, we actually found a change of ~4%, clearly greater than the expected effect of CBV alone and suggesting that other physiologic changes may contribute to the observed signal. While on the plane during one of his travels, *Peter van Zijl* derived the theory that CBF should contribute at short TR and Manus Donahue, a new graduate student in Dr. van Zijl's lab, conducted systematic experiments and simulations to confirm this (Donahue et al., 2006). In addition to CBF, another confound is the danger of actual inflow effects during the experiments. In trying to overcome this confounding flow contribution, *Hanzhang Lu* tried to apply a post-saturation RF pulse after the echo acquisition to reset the blood magnetization, but this was found to only partially correct for the spurious effects (Lu, 2008). Fortunately, this effect is small when using body coils for excitation (Donahue et al., 2009), which are currently available on most human scanners and also some animal scanners. However, in order to avoid the CBF effects and potential inflow effects it is recommended to use TR values of 5 s or more when predominant CBV contrast is required in VASO fMRI.

A second limitation of VASO fMRI is its lower sensitivity compared to BOLD. The main constraint is the unwanted tissue suppression (to about 20% of the equilibrium magnetization) by the inversion pulse (Lu et al., 2003). As a result, the contrast-to-noise ratio (CNR) of the original VASO fMRI approach is about 1/5–1/3 of that of BOLD (Lu et al., 2003). The use of higher field strength is obviously a possible solution to increase signal to noise ratio (SNR) and CNR. We found that VASO CNR by 65% when comparing 3T to 1.5T, resulting in 143% more activated voxels (Lu and van Zijl, 2005). As we move to even higher field, however, several factors may counteract the improvement in intrinsic SNR. First, at higher field, the T₁ difference between blood and tissue becomes less pronounced (Tsekos et al., 1998), reducing the fraction of remaining tissue at the blood nulling time

point. Second, the BOLD effect is considerably increased at higher field which will offset the CBV effect (Yacoub et al., 2005). Finally, the sensitivity of the BOLD method is also augmented at higher field (Duong et al., 2003). Thus, when it comes to the comparison between VASO and BOLD, the sensitivity of VASO is still a lot lower regardless of which field strength they are compared at (Lu and van Zijl, 2005). To confront this problem, it would be necessary to increase the tissue signal during nulling. One way to do this is by artificially speeding up the longitudinal magnetization recovery, which can be done by applying magnetization transfer pulses before or during inversion (Hua et al., 2009). This leads to CNR enhancements by 44% and 36%, respectively. An even better solution would be to prevent the static tissue spins from experiencing the inversion pulses, thereby eliminating the SNR loss of 80% as occurring in the original VASO technique. In order to achieve this, Hua et al. recently proposed an inflow-based VASO (iVASO) technique in which a second slice selective inversion pulse is applied on the brain slice immediately following the first nonselective inversion. As a consequence, only the incoming blood spins experience nulling while the static tissue spins remain effectively uninverted (Hua et al., 2011a). This approach, although initially not intended for it, lead to the possibility to measure arterial and arteriolar CBV effects. If the inversion time (together with the corresponding TR) is chosen properly, one can allow the inverted spins to reach arterial and arteriolar vasculature at the time of nulling, but not capillary/tissue/veins. Using the proposed scheme, the authors showed that the SNR of iVASO was as much as three-fold that of the original VASO, offering great potential for the technique to compete with conventional BOLD in terms of sensitivity (Hua et al., 2011a). A confounding factor of this technique is that the effect depends on arterial and tissue transit times of the inverted spins, which may lead to uncertainty of the measured CBV changes under pathological conditions. On the other hand, since vasodilatation during brain activation primarily takes place in arterial and arteriolar vessels, the iVASO changes allow this to be monitored in humans and compare the effects to optical studies in animals. An additional advantage is that absolute arterial/arteriolar CBV can be measured (Donahue et al., 2010; Hua et al., 2011b).

One of the confounding factors in interpreting the VASO fMRI signal is the contribution of CSF. In the original VASO model, the voxel was presumed to contain two compartments only, tissue and blood (Lu et al., 2003). Thus, an increase in blood volume within a voxel would predict a decrease in tissue volume by the same amount. The brain, however, also contains CSF, which has a long T1 (about 4s at typical fields in the clinic) and is expected to have a negative magnetization at the blood nulling time. CSF contributions may influence the interpretation of VASO fMRI in two ways: impact on baseline VASO signal and impact on activation-induced signal change due to potential volume decrease in CSF itself. These issues have been systematically investigated by a number of researchers (Donahue et al., 2006; Jin and Kim, 2010; Piechnik et al., 2009; Scouten and Constable, 2007, 2008). First, the presence of CSF will lower the baseline VASO signal as its negative magnetization will offset some of the positive tissue signals. Detailed theoretical simulations have shown that, even in the absence of activation-induced changes, this effect may cause an over-estimation of $\Delta\text{CBV}/\text{CBV}$ (Scouten and Constable, 2007). Moreover, several studies have suggested that CSF volume may show a decrease upon activation (presumably to accommodate the CBV increase) (Donahue et al., 2006; Jin and Kim, 2010; Piechnik et al., 2009; Scouten and Constable, 2008). This effect tends to cause a signal increase in VASO fMRI, potentially overriding the VASO effect when occurring concurrently with the hypothesized tissue-shrinking effect. This effect may explain the absence of VASO signal during neural activation or hypercapnia challenges in regions with high CSF fraction such as the primary auditory cortex and insula (Scouten and Constable, 2007, 2008).

Sufficient spatial coverage is also a potential challenge for VASO fMRI. Since the method is based on blood nulling and, by theory, there is only one nulling point during longitudinal

relaxation, it appears that only one excitation RF pulse can be applied during each TR. As a result, VASO fMRI is typically acquired in single-slice mode (Lu et al., 2003), presenting a practical restriction when using this technique for neuroscience or clinical applications. Fortunately, there are ways around this. To overcome this limitation, we first developed a scheme called Multiple-Acquisition-with-Global-Inversion-Cycling (MAGIC), in which the longitudinal magnetization that has just crossed the zero point is inverted again by another inversion pulse, causing another zero-crossing to occur. When using a series of such pulses, multiple nulling points can be achieved allowing multiple slices to be acquired in one TR (Lu et al., 2004c). This method has been recently extended by Scouten and Constable (2007, 2008) to acquire 21 slices within one TR, allowing whole brain coverage. However, a disadvantage is that the multiple slices acquired have a pattern of gradual signal decrease, rendering it difficult to perform image realignment. With recent advances in image acquisition and reconstruction approaches as well as hardware improvement, it is now possible to acquire 3D volumes after a slab-selective RF excitation. Poser and Norris showed that single-shot 3D GRASE can be used for VASO acquisition, which covered 20 slices with $3.5 \times 3.5 \times 5 \text{ mm}^3$ spatial resolution (Poser and Norris, 2011). Reliable activations were observed during a cognitive paradigm, Stroop color-word matching task. In addition, several other imaging technologies also have the potential to be used for improving spatial coverage in VASO. A common feature of these methods is that they allow 3D/multi-slice acquisition following one RF pulse. One such technique is called MR inverse imaging, which applies frequency and phase encoding in two spatial directions similar to conventional EPI, while spatial discrimination in the third direction is achieved by solving the inverse problem utilizing information from all array channels (Lin et al., 2008b). With this technique, whole-brain acquisition can be achieved following a single RF excitation within about 100 ms (Lin et al., 2008b). Another interesting acquisition technique is called multiplexed-EPI (M-EPI), which combines two forms of fast imaging approaches, namely temporal multiplexing (m) utilizing simultaneous echo refocused EPI and spatial multiplexing (n) with multi-banded RF pulses (Feinberg et al., 2010). Therefore, $m \times n$ images can be acquired with a single-shot acquisition. These cutting-edge technologies, although not yet applied in VASO fMRI, offer great potentials for expanding the utility of VASO imaging in future studies.

Finally, VASO fMRI reflects changes in total CBV. However, in the context of BOLD signal mechanism, venous CBV may be more relevant as deoxyhemoglobin is primarily found in capillary and venous vessels (Buxton et al., 1998; Davis et al., 1998; Hoge et al., 1999; Mandeville et al., 1999; Ogawa et al., 1993; van Zijl et al., 1998). To estimate venous CBV changes during activation in (partially) deoxygenated regions, one can consider conducting a combined VASO (Lu et al., 2003) and iVASO (Hua et al., 2011a) (sensitive to arterial CBV changes) experiment, the subtraction of which is expected to provide information on capillary and venous CBV. One may also directly assess venous CBV changes using other advanced techniques such as Venous refocusing for volume estimation (VERVE) fMRI, which exploits the refocusing-time dependence of the venous signal to specifically image venous CBV (Chen and Pike, 2009; Stefanovic and Pike, 2005) assuming the oxygenation of venous blood is known.

Conclusions

VASO fMRI provides a non-invasive, dynamic, and repeatable method for mapping brain function based on changed in CBV without the need for a contrast agent. This technique can be easily applied in conjunction with other fMRI approaches such as BOLD and ASL fMRI, providing a better understanding of metabolic and hemodynamic changes during neural activation and also improving the quantification of extravascular BOLD signals. With

technical advances in image acquisition and reconstruction, VASO fMRI has the potential to be a useful tool in neuroscience and clinical applications.

Acknowledgments

This study was supported in part by NIH grants R01 MH084021 (to HL), R01 NS067015 (to HL), and P41 RR15241 (to PvZ).

References

- Alsop DC, Detre JA. Reduced transit-time sensitivity in noninvasive magnetic resonance imaging of human cerebral blood flow. *J Cereb Blood Flow Metab.* 1996; 16:1236–1249. [PubMed: 8898697]
- Belliveau JW, Kennedy DN Jr, McKinstry RC, Buchbinder BR, Weisskoff RM, Cohen MS, Vevea JM, Brady TJ, Rosen BR. Functional mapping of the human visual cortex by magnetic resonance imaging. *Science.* 1991; 254:716–719. [PubMed: 1948051]
- Boxerman JL, Hamberg LM, Rosen BR, Weisskoff RM. MR contrast due to intravascular magnetic susceptibility perturbations. *Magn Reson Med.* 1995; 34:555–566. [PubMed: 8524024]
- Buxton RB, Wong EC, Frank LR. Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. *Magn Reson Med.* 1998; 39:855–864. [PubMed: 9621908]
- Calamante F, Thomas DL, Pell GS, Wiersma J, Turner R. Measuring cerebral blood flow using magnetic resonance imaging techniques. *J Cereb Blood Flow Metab.* 1999; 19:701–735. [PubMed: 10413026]
- Chen JJ, Pike GB. BOLD-specific cerebral blood volume and blood flow changes during neuronal activation in humans. *NMR Biomed.* 2009; 22:1054–1062. [PubMed: 19598180]
- Davis TL, Kwong KK, Weisskoff RM, Rosen BR. Calibrated functional MRI: mapping the dynamics of oxidative metabolism. *Proc Natl Acad Sci U S A.* 1998; 95:1834–1839. [PubMed: 9465103]
- Detre JA, Leigh JS, Williams DS, Koretsky AP. Perfusion imaging. *Magn Reson Med.* 1992; 23:37–45. [PubMed: 1734182]
- Donahue MJ, Hua J, Pekar JJ, van Zijl PC. Effect of inflow of fresh blood on vascular-space-occupancy (VASO) contrast. *Magn Reson Med.* 2009; 61:473–480. [PubMed: 19161167]
- Donahue MJ, Lu H, Jones CK, Edden RA, Pekar JJ, van Zijl PC. Theoretical and experimental investigation of the VASO contrast mechanism. *Magn Reson Med.* 2006; 56:1261–1273. [PubMed: 17075857]
- Donahue MJ, Sideso E, MacIntosh BJ, Kennedy J, Handa A, Jezzard P. Absolute arterial cerebral blood volume quantification using inflow vascular-space-occupancy with dynamic subtraction magnetic resonance imaging. *J Cereb Blood Flow Metab.* 2010; 30:1329–1342. [PubMed: 20145656]
- Duck FA. Nuclear magnetism of tissue. 1990:279–317.
- Duong TQ, Yacoub E, Adriany G, Hu X, Ugurbil K, Kim SG. Microvascular BOLD contribution at 4 and 7 T in the human brain: gradient-echo and spin-echo fMRI with suppression of blood effects. *Magn Reson Med.* 2003; 49:1019–1027. [PubMed: 12768579]
- Edelman RR, Siewert B, Darby DG, Thangaraj V, Nobre AC, Mesulam MM, Warach S. Qualitative mapping of cerebral blood flow and functional localization with echo-planar MR imaging and signal targeting with alternating radio frequency. *Radiology.* 1994; 192:513–520. [PubMed: 8029425]
- Feinberg DA, Moeller S, Smith SM, Auerbach E, Ramanna S, Glasser MF, Miller KL, Ugurbil K, Yacoub E. Multiplexed echo planar imaging for sub-second whole brain FMRI and fast diffusion imaging. *PLoS One.* 2010; 5:e15710. [PubMed: 21187930]
- Glielmi CB, Schuchard RA, Hu XP. Estimating cerebral blood volume with expanded vascular space occupancy slice coverage. *Magn Reson Med.* 2009; 61:1193–1200. [PubMed: 19253363]
- Golay X, Stuber M, Pruessmann KP, Meier D, Boesiger P. Transfer insensitive labeling technique (TILT): application to multislice functional perfusion imaging. *J Magn Reson Imaging.* 1999; 9:454–461. [PubMed: 10194717]

- Gu H, Lu H, Ye FQ, Stein EA, Yang Y. Noninvasive quantification of cerebral blood volume in humans during functional activation. *Neuroimage*. 2006; 30:377–387. [PubMed: 16278086]
- Harrison RV, Harel N, Panesar J, Mount RJ. Blood capillary distribution correlates with hemodynamic-based functional imaging in cerebral cortex. *Cereb Cortex*. 2002; 12:225–233. [PubMed: 11839597]
- Hoge RD, Atkinson J, Gill B, Crelier GR, Marrett S, Pike GB. Linear coupling between cerebral blood flow and oxygen consumption in activated human cortex. *Proc Natl Acad Sci U S A*. 1999; 96:9403–9408. [PubMed: 10430955]
- Hoogenraad FG, Pouwels PJ, Hofman MB, Reichenbach JR, Sprenger M, Haacke EM. Quantitative differentiation between BOLD models in fMRI. *Magn Reson Med*. 2001; 45:233–246. [PubMed: 11180431]
- Hua J, Donahue MJ, Zhao JM, Grgac K, Huang AJ, Zhou J, van Zijl PC. Magnetization transfer enhanced vascular-space-occupancy (MT-VASO) functional MRI. *Magn Reson Med*. 2009; 61:944–951. [PubMed: 19215043]
- Hua J, Qin Q, Donahue MJ, Zhou J, Pekar JJ, van Zijl PC. Inflow-based vascular-space-occupancy (iVASO) MRI. *Magn Reson Med*. 2011a; 66:40–56. [PubMed: 21695719]
- Hua J, Qin Q, Pekar JJ, van Zijl PC. Measurement of absolute arterial cerebral blood volume in human brain without using a contrast agent. *NMR Biomed*. 2011b In-press; doi: 10.1002/nbm.1693.
- Hua J, Stevens RD, Huang AJ, Pekar JJ, van Zijl PC. Physiological origin for the BOLD poststimulus undershoot in human brain: vascular compliance versus oxygen metabolism. *J Cereb Blood Flow Metab*. 2011c; 31:1599–1611. [PubMed: 21468090]
- Iadecola C. Intrinsic signals and functional brain mapping: caution, blood vessels at work. *Cereb Cortex*. 2002; 12:223–224. [PubMed: 11839596]
- Jin T, Kim SG. Improved cortical-layer specificity of vascular space occupancy fMRI with slab inversion relative to spin-echo BOLD at 9.4 T. *Neuroimage*. 2008; 40:59–67. [PubMed: 18249010]
- Jin T, Kim SG. Change of the cerebrospinal fluid volume during brain activation investigated by T(1rho)-weighted fMRI. *Neuroimage*. 2010; 51:1378–1383. [PubMed: 20338251]
- Kim SG. Quantification of relative cerebral blood flow change by flow-sensitive alternating inversion recovery (FAIR) technique: application to functional mapping. *Magn Reson Med*. 1995; 34:293–301. [PubMed: 7500865]
- Kuschinsky, W. Regulation of cerebral blood flow: an overview. In: Mraovitch, S.; Sercombe, R., editors. *Neurophysiological basis of cerebral blood flow control: an introduction*. Johns Libbey & Company Ltd; London: 1996. p. 245-262.
- Lin AL, Fox PT, Yang Y, Lu H, Tan LH, Gao JH. Evaluation of MRI models in the measurement of CMRO₂ and its relationship with CBF. *Magn Reson Med*. 2008a; 60:380–389. [PubMed: 18666102]
- Lin AL, Fox PT, Yang Y, Lu H, Tan LH, Gao JH. Time-dependent correlation of cerebral blood flow with oxygen metabolism in activated human visual cortex as measured by fMRI. *Neuroimage*. 2009; 44:16–22. [PubMed: 18804541]
- Lin FH, Witzel T, Mandeville JB, Polimeni JR, Zeffiro TA, Greve DN, Wiggins G, Wald LL, Belliveau JW. Event-related single-shot volumetric functional magnetic resonance inverse imaging of visual processing. *Neuroimage*. 2008b; 42:230–247. [PubMed: 18538587]
- Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. *Nature*. 2001; 412:150–157. [PubMed: 11449264]
- Lu, H. Magnetization “reset” for Non-steady-state blood spins in Vascular-Space-Occupancy (VASO) fMRI. *Proc. of 16th Annual Meeting of ISMRM*; Toronto, Canada. 2008. p. 406
- Lu H, Basso G, Serences JT, Yantis S, Golay X, van Zijl PC. Retinotopic mapping in the human visual cortex using vascular space occupancy-dependent functional magnetic resonance imaging. *Neuroreport*. 2005a; 16:1635–1640. [PubMed: 16189469]
- Lu H, Clingman C, Golay X, van Zijl PC. Determining the longitudinal relaxation time (T₁) of blood at 3.0 Tesla. *Magn Reson Med*. 2004a; 52:679–682. [PubMed: 15334591]
- Lu, H.; Donahue, MJ.; Jones, CK.; van Zijl, PC. Spatial Characteristics of VASO fMRI at Ultra-high Resolution. *Proc. of 13th Annual Meeting of ISMRM*; Miami, FL. 2005b. p. 27

- Lu H, Golay X, Pekar JJ, Van Zijl PC. Sustained poststimulus elevation in cerebral oxygen utilization after vascular recovery. *J Cereb Blood Flow Metab.* 2004b; 24:764–770. [PubMed: 15241184]
- Lu H, Golay X, Pekar JJ, van Zijl PC. Functional magnetic resonance imaging based on changes in vascular space occupancy. *Magn Reson Med.* 2003; 50:263–274. [PubMed: 12876702]
- Lu H, Golay X, van Zijl PC. Intervoxel heterogeneity of event-related functional magnetic resonance imaging responses as a function of T(1) weighting. *Neuroimage.* 2002; 17:943–955. [PubMed: 12377168]
- Lu H, van Zijl PC. Experimental measurement of extravascular parenchymal BOLD effects and tissue oxygen extraction fractions using multi-echo VASO fMRI at 1.5 and 3.0 T. *Magn Reson Med.* 2005; 53:808–816. [PubMed: 15799063]
- Lu H, van Zijl PC, Hendrikse J, Golay X. Multiple acquisitions with global inversion cycling (MAGIC): a multislice technique for vascular-space-occupancy dependent fMRI. *Magn Reson Med.* 2004c; 51:9–15. [PubMed: 14705039]
- Mandeville JB, Marota JJ, Ayata C, Zaharchuk G, Moskowitz MA, Rosen BR, Weisskoff RM. Evidence of a cerebrovascular postarteriole windkessel with delayed compliance. *J Cereb Blood Flow Metab.* 1999; 19:679–689. [PubMed: 10366199]
- Mandeville JB, Marota JJ, Kosofsky BE, Keltner JR, Weissleder R, Rosen BR, Weisskoff RM. Dynamic functional imaging of relative cerebral blood volume during rat forepaw stimulation. *Magn Reson Med.* 1998; 39:615–624. [PubMed: 9543424]
- Ogawa S, Menon RS, Tank DW, Kim SG, Merkle H, Ellermann JM, Ugurbil K. Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. *Biophys J.* 1993; 64:803–812. [PubMed: 8386018]
- Piechnik SK, Evans J, Bary LH, Wise RG, Jezzard P. Functional changes in CSF volume estimated using measurement of water T2 relaxation. *Magn Reson Med.* 2009; 61:579–586. [PubMed: 19132756]
- Poser BA, Norris DG. Measurement of activation-related changes in cerebral blood volume: VASO with single-shot HASTE acquisition. *Magma.* 2007; 20:63–67. [PubMed: 17318490]
- Poser BA, Norris DG. 3D single-shot VASO using a Maxwell gradient compensated GRASE sequence. *Magn Reson Med.* 2009; 62:255–262. [PubMed: 19319900]
- Poser BA, Norris DG. Application of whole-brain CBV-weighted fMRI to a cognitive stimulation paradigm: robust activation detection in a stroop task experiment using 3D GRASE VASO. *Hum Brain Mapp.* 2011; 32:974–981. [PubMed: 20578174]
- Scouten A, Constable RT. Applications and limitations of whole-brain MAGIC VASO functional imaging. *Magn Reson Med.* 2007; 58:306–315. [PubMed: 17654574]
- Scouten A, Constable RT. VASO-based calculations of CBV change: accounting for the dynamic CSF volume. *Magn Reson Med.* 2008; 59:308–315. [PubMed: 18228581]
- Shen Y, Kauppinen RA, Vidyasagar R, Golay X. A functional magnetic resonance imaging technique based on nulling extravascular gray matter signal. *J Cereb Blood Flow Metab.* 2009; 29:144–156. [PubMed: 18728677]
- Stefanovic B, Pike GB. Venous refocusing for volume estimation: VERVE functional magnetic resonance imaging. *Magn Reson Med.* 2005; 53:339–347. [PubMed: 15678548]
- Tsekos NV, Zhang F, Merkle H, Nagayama M, Iadecola C, Kim SG. Quantitative measurements of cerebral blood flow in rats using the FAIR technique: correlation with previous iodoantipyrine autoradiographic studies. *Magn Reson Med.* 1998; 39:564–573. [PubMed: 9543418]
- Turner R. How much cortex can a vein drain? Downstream dilution of activation-related cerebral blood oxygenation changes. *Neuroimage.* 2002; 16:1062–1067. [PubMed: 12202093]
- van Bruggen N, Busch E, Palmer JT, Williams SP, de Crespigny AJ. High-resolution functional magnetic resonance imaging of the rat brain: mapping changes in cerebral blood volume using iron oxide contrast media. *J Cereb Blood Flow Metab.* 1998; 18:1178–1183. [PubMed: 9809506]
- van Zijl PC, Eleff SM, Ulatowski JA, Oja JM, Ulug AM, Traystman RJ, Kauppinen RA. Quantitative assessment of blood flow, blood volume and blood oxygenation effects in functional magnetic resonance imaging. *Nat Med.* 1998; 4:159–167. [PubMed: 9461188]

- van Zijl PC, Hua J, Lu H. The BOLD post-stimulus undershoot, one of the most debated issues in fMRI. *Neuroimage*, TBD. 2012
- Wong EC, Buxton RB, Frank LR. Quantitative imaging of perfusion using a single subtraction (QUIPSS and QUIPSS II). *Magn Reson Med*. 1998; 39:702–708. [PubMed: 9581600]
- Wu CW, Chuang KH, Wai YY, Wan YL, Chen JH, Liu HL. Vascular space occupancy-dependent functional MRI by tissue suppression. *J Magn Reson Imaging*. 2008; 28:219–226. [PubMed: 18581345]
- Wu WC, Buxton RB, Wong EC. Vascular space occupancy weighted imaging with control of residual blood signal and higher contrast-to-noise ratio. *IEEE Trans Med Imaging*. 2007; 26:1319–1327. [PubMed: 17948723]
- Yablonskiy DA, Haacke EM. Theory of NMR signal behavior in magnetically inhomogeneous tissues: the static dephasing regime. *Magn Reson Med*. 1994; 32:749–763. [PubMed: 7869897]
- Yacoub E, Van De Moortele PF, Shmuel A, Ugurbil K. Signal and noise characteristics of Hahn SE and GE BOLD fMRI at 7 T in humans. *Neuroimage*. 2005; 24:738–750. [PubMed: 15652309]
- Yang Y, Gu H, Stein EA. Simultaneous MRI acquisition of blood volume, blood flow, and blood oxygenation information during brain activation. *Magn Reson Med*. 2004; 52:1407–1417. [PubMed: 15562477]
- Zhong J, Kennan RP, Fulbright RK, Gore JC. Quantification of intravascular and extravascular contributions to BOLD effects induced by alteration in oxygenation or intravascular contrast agents. *Magn Reson Med*. 1998; 40:526–536. [PubMed: 9771569]

Figure 1.

Results from the very first VASO experiment. Plots in (a) and (b) show MR signal time courses during visual stimulation using BOLD and VASO pulse sequences, respectively. The signals were averaged in a region-of-interest (ROI) in the occipital lobe. The ROI was defined as activated voxels in the BOLD scan. The VASO scan did not yield any significant voxels in this case because, with the long TE used (50 ms), the extravascular BOLD effect has partly offset the CBV effect. However, a small signal drop during stimulation was still noticeable in the VASO time course, which can be further confirmed by an anti-correlation ($cc=-0.37$) with the stimulus paradigm. Red symbols indicate time points during visual stimulation.

Figure 2. VASO fMRI activation map using visual stimulation (*29.6 sec ON, 29.6 sec OFF*). The panel on the right shows the averaged time course of these voxels.

Figure 3.

Spatial specificity of VASO fMRI. Panels (a) and (b) show activation maps using VASO and BOLD, respectively, in human visual cortex. Panels (c), (d) and (e) show activation maps using slab-selective (SI) VASO, contrast agent, and spin-echo BOLD, respectively, in cat visual cortex. Panel (f) shows signal amplitude as a function of distance to the cortical surface. VASO and contrast agent based fMRI show activation signals peaked around layer IV of the cortex whereas the BOLD signal was widespread across all layers. (Panels c through f were adapted from Jin and Kim, 2008, with permission).